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Research Paper

Performance of pistachio (*Pistacia vera* L.) in warming Mediterranean orchards

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ABSTRACT

Woody perennial species from temperate regions fall dormant during the cold winter season to avoid unfavourable conditions. To break out of dormancy and eventually flower, they must fulfil cultivar-specific chilling and heat requirements. Phenology analysis can clarify the climatic requirements of tree cultivars and thus provide critical information to ensure the future viability of orchards in warm growing regions, where warmer winters are expected as a result of climate change. We used Partial Least Squares (PLS) regression to correlate first bloom dates of 4 local and 3 foreign pistachio (*Pistacia vera* L.) cultivars with daily chill and heat accumulation (quantified with the Dynamic Model and Growing Degree Hours Model, respectively) for 18-year records (1997–2016) from Sfax, Tunisia. PLS outputs allowed delineation of the chilling phase, during which high chill accumulation was correlated to early bloom, and the forcing phase, when this was true for high heat accumulation. Both phases showed discontinuities. During September and October, high heat accumulation appeared to first have a bloom-delaying effect, followed by a bloom-advancing effect, indicating that temperature during dormancy induction may affect bloom dates. Chilling requirements were estimated between 32.1 ± 2.3 and 33.3 ± 2.2 Chill Portions and heat requirements between 9974 ± 198 and 12,738 ± 235 Growing Degree Hours. This study revealed limitations of the Dynamic Model, which is often considered the most accurate among commonly used models, in the warm Tunisian climate. High temperatures during the chilling phase had a significant bloom-delaying effect on all pistachio cultivars. Low chill accumulation was related to very low yields and associated with zero production in 1995, 2001 and 2007. Low flowering percentage, high bud fall percentage, long and inhomogeneous bloom, and co-occurrence of several phenological stages on the same branch were symptoms of lack of chill in 2016.

1. Introduction

Pistachios are an important nut crop that is widely cultivated in Mediterranean climates. Even though the global production of in-shell pistachios dropped from 638 to 524 thousand metric tons between 2014 and 2015, growers have roughly doubled production since 2004 (International Nut and Dried Fruit Council, 2016, 2015). Much of this expansion has occurred in places where winter temperatures are substantially higher than in the species' centre of origin in Central Asia or in its traditional production regions. Expanding the production of temperate-zone tree species into warm climates may lead to a mismatch between tree physiology and climatic conditions (Erez, 2000). Woody perennial species that evolved in cold-winter regions fall dormant during the cold season to reduce exposure of sensitive growing tissue to unfavourable conditions (Atkinson et al., 2013; Campoy et al., 2011; Faust et al., 1997; Jones et al., 2013). In order to break out of their dormant state, most such species require fulfilment of a chilling requirement, which conditions them to resume growth, produce leaves and eventually bloom and develop fruits, as their environment warms (Jones et al., 2015). The timing of each of these developmental stages is understood to further depend on heat requirements that are specific to each stage and vary across tree cultivars (Luedeling, 2012). Especially the compulsory chilling requirements make temperate-zone tree species potentially vulnerable to climate change, because rising temperatures

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may reduce available chill (Luedeling et al., 2011), causing physiological disorders and threatening the productivity and economic viability of orchards (Erez, 2000).

While this challenge is a concern in many pistachio growing regions around the world, it is particularly daunting for Tunisia, which features some of the warmest pistachio orchards on the planet (Elloumi et al., 2013). Here, chill levels are likely already marginal for pistachio production, and climate projections for the coming decades indicate continued warming (Schilling et al., 2012). This is expected to further reduce available chill (Luedeling et al., 2011), which may jeopardize orchard productivity and sustainability (Darbyshire et al., 2011; Ghrab et al., 2014a). Lack of sufficient chill can have severe consequences (Atkinson et al., 2013), causing physiological symptoms such as shifts in the timing of developmental stages (Miller-Rushing et al., 2007; Ramírez and Kallarackal, 2015), irregular or delayed budbreak, abscission of floral buds, altered reproductive morphology, poor fruit set and changes in vegetative growth (Atkinson et al., 2013). Most of these effects reduce yield and fruit or nut quality (Legave et al., 2013; Lopez and Dejong, 2007). In pistachio, nut quality responds to climate conditions (Doster et al., 2001, 1999; Tajabadipour et al., 2006). Where low chill and changes in winter temperatures affect the timing of developmental stages, changing nutrient demand patterns may cause nutritional imbalances that can lead to early dehiscence, and empty or cracked shells (Hosseinifard and Panahi, 2006; Khezri et al., 2010). In dioecious species like pistachio, changing dormant-season conditions could reduce pollination rates, if male and female trees lose their flowering synchrony (Luedeling et al., 2009a). The large array of production problems that can arise from a mismatch between local climate and tree requirements makes the selection of appropriate cultivars a crucial prerequisite to economically viable fruit and nut production. In making such choices, orchard managers, especially those operating in warm climates, should consider the potential impact of climate change.

Plant phenology is a sensitive indicator of climate variation and change (Fu et al., 2015). Analysis of bloom and leafing dates may therefore allow insights into tree responses to climate and provide useful information for the development of adaptation strategies (Valentini et al., 2001). Such strategies may include the application of rest-breaking agents or shifts towards better-adapted species or cultivars. As a first step towards this goal, phenology analysis can help clarify the climatic requirements of tree cultivars (Benmoussa et al., 2017), which is critical information for preparing orchards in warm growing regions for warming winters (Gao et al., 2012; Jones et al., 2015; Naor et al., 2003).

Precise quantification of chilling requirements is hampered by the poor performance of commonly used chill models, such as the Chilling Hours Model (Weinberger, 1950) or the Utah Model (Richardson et al., 1974), in warm growing regions (Benmoussa et al., 2017; Elloumi et al., 2013; Ghrab et al., 2014a; Pérez et al., 2008). Chilling requirements determined with such models cannot be assumed to be valid across locations (Luedeling and Brown, 2011) or to remain accurate as temperature increases (Luedeling et al., 2009b). This makes it difficult to use information generated by past studies in regions with different climatic conditions. Among such studies, which have been conducted in Turkey (Küden et al., 1995), Iran (Afshari et al., 2009; Esmaeilizadeh et al., 2006; Rahemi and Pakkish, 2009) and Australia (Zhang and Taylor, 2011), chilling requirements of various pistachio cultivars ranged between 500 and 1400 Chilling Hours or between 500 and 1123 Chill Units (of the Utah Model). These estimates are clearly not applicable in Tunisia, where pistachios are successfully grown, but available chill does not even come close to these alleged requirements (Elloumi et al., 2013). The most promising model for more widely applicable estimates is the Dynamic Model (Erez and Fishman, 1998; Fishman et al., 1987a, 1987b), according to which 'Sirora' pistachios in Australia require around 60 Chill Portions (Zhang and Taylor, 2011). This is also far more than what is available in Tunisia, where, however, this cultivar is not grown. The only available information obtained under Tunisian conditions was produced for the widespread 'Mateur' cultivar, for which chilling requirements were estimated by Salhi et al. (2014) (600 Chill Units) and Elloumi et al. (2013) (206 Chilling Hours, 539 Chill Units or 36 Chill Portions). Information on heat requirements is similarly inconsistent, with studies from Turkey, Iran and Australia indicating a need for 8852–17,297 Growing Degree Hours (Küden et al., 1995; Rahemi and Pakkish, 2009; Zhang and Taylor, 2011) as opposed to 12,000 determined by Salhi et al. (2014) for Tunisia.

Recent warm winters in Tunisia raise concerns about tree responses to future temperature increases. There is thus an urgent need for locally appropriate information on tree responses to temperature, which can help anticipate problems and develop adaptation strategies.

To this end, we investigated the relationship between temperature and bloom dates for 7 pistachio cultivars of local and foreign origin, using an 18-year record from the Sfax region of Tunisia. We used Partial Least Squares (PLS) regression (Luedeling and Gassner, 2012) to correlate pistachio bloom dates with daily chill and heat accumulation and to delineate temperature response phases. This method has been used in several recent studies (Benmoussa et al., 2017; Guo et al., 2015a; Luedeling et al., 2013b) to determine the chilling and forcing periods of several fruit and nut species, including successful applications under warm conditions. We explored tree responses to temperature variation during these periods and evaluated correlations between agroclimatic metrics, phenology and productivity.

2. Materials and methods

2.1. Study site and cultivars

This study was carried out in the Olive Institute's experimental station in Taous (located at 34°56′08″N; 10°36′54″E), in the Sfax region of central Tunisia. The 3-ha experimental orchard is characterized by deep sandy soil, on which trees are grown under rain-fed conditions, using standard horticultural management practices typical of the region. The Sfax region is characterized by a semi-arid Mediterranean climate, with annual precipitation of 204 mm and reference evapotranspiration (ET_0) of 1340 mm.

Forty-year-old trees of 4 local and 3 foreign pistachio cultivars grafted on *Pistacia vera* were used for this study. The local cultivars were 'Mateur' (from northern Tunisia), 'El-Guetar' (central Tunisia), 'Nouri' (Sfax, Tunisia) and 'Meknassy' (central Tunisia). The foreign cultivars were 'Kerman' (from Rafsanjan, Iran and widely grown in California, USA), 'Ohadi' (Iran) and 'Red Aleppo' (Syria).

2.2. Phenology, yield and weather data

Pistachio tree phenology was observed from 1997 to 2016, with first bloom dates recorded when 5% of flower buds had reached the 'Brown Flowers Cluster' stage – one of several pistachio flower opening stages described by Zhang (2006). For the 'Mateur' cultivar, mean yields (in kg per tree) were recorded from 1983 to 2015.

Daily minimum and maximum temperatures from 1973 to 2016 were obtained from the Sfax El-Maou station and daily extreme temperatures in the orchard determined according to transfer functions developed by Benmoussa et al. (2017). Hourly temperatures, which were required for calculating chill and heat accumulation with common chill and heat models, were calculated from minimum and maximum temperatures using the chillR package (Luedeling, 2016) and based on procedures described by Almorox et al. (2005); Linvill (1990) and Spencer (1971).

2.3. Chilling and forcing phases, and chilling and heat requirements

Based on hourly temperatures, we calculated daily chill and heat accumulation between 1997 and 2016. Even though multiple chilling models are commonly used (e.g. the Chilling Hours or Utah Models), we based all our results on the Dynamic Model (which quantifies chill in Chill Portions – CP) (Erez and Fishman, 1998; Fishman et al., 1987a, 1987b), because its superior accuracy in warm climates has been demonstrated many times (Benmoussa et al., 2017; Elloumi et al., 2013; Ghrab et al., 2014a; Guo et al., 2015b, 2013; Luedeling, 2012; Luedeling et al., 2009b; Luedeling and Brown, 2011; Pérez et al., 2008), including for pistachio (Elloumi et al., 2013; Zhang and Taylor, 2011). Heat accumulation was quantified with the Growing Degree Hours Model (Growing Degree Hours – GDH) (Anderson et al., 1986). Chilling and forcing phases were determined by applying Partial Least Squares Regression (PLS) (Luedeling and Gassner, 2012). From 1997 to 2016, first bloom dates of pistachio cultivars were correlated with daily chill and heat accumulation. The Variable Importance in the Projection (VIP) and standardized model coefficients are the two major outputs of PLS regression (Guo et al., 2015a, 2015b, 2013; Luedeling et al., 2013a). Chilling and forcing phases were defined based on VIP scores and model coefficients determined for daily chill and heat accumulation rates. Daily accumulation rates were classified as important, when their VIP score was greater than 0.8 and they were likely to belong to the chilling or forcing period, when they had negative model coefficients for chill or heat accumulation, respectively. Median bloom dates were considered as the end of the heat period. Through this choice, we did not capture all forcing days in late-bloom years, but we limited inclusion of heat units after bloom in early-bloom years (which could not possibly affect bloom). Since a single date for the end of the forcing period needed to be defined − to correspond to the single date PLS regression indicated for the beginning of forcing − use of the median constituted a reasonable compromise. All daily chill and heat accumulations during chilling and forcing periods were summed up and the means of these sums across all years used as estimates of the chilling and heat requirements of pistachio cultivars. The standard error was computed as a measure of uncertainty. All analyses were done in the R programming environment (version 3.2.2) (R Development Core Team, 2015), using the chillR package (version 0.64.4) (Luedeling, 2016).

2.4. Correlations between bloom date, yield and chill accumulation

For each cultivar, chill accumulation during the chilling period was calculated using the Dynamic Model and correlated with bloom dates from 1997 to 2016. For the 'Mateur' cultivar, correlations were also determined between chill accumulation and crop yields (1983–2015) and between bloom dates and crop yields (1997–2015). All correlations were tested for statistical significance using Spearman's non-parametric rank correlation test.

2.5. Field survey

A field survey was carried out in 2015 and 2016 for all pistachio cultivars expect 'El-Guetar'. For each cultivar, 3 trees were chosen and for each tree 4 shoots were selected and tagged. For each tagged shoot, we recorded the number of flower buds and their phenological stages once a week from March to May according to pistachio flower opening stages defined by Zhang (2006). At the end of the bloom period and before fruit set, we calculated the percentage of buds in the dormant stage, the percentage of buds in the budbreak stage, the percentage of bud fall and the percentage of flowering.

3. Results

3.1. Bloom dates and chilling and forcing periods

Pistachio bloom dates displayed substantial interannual variation, but showed systematic effects of cultivar origin, with foreign cultivars always blooming later than local ones (Table 1). For local cultivars, the median bloom dates were between 10th and 14th April, with bloom dates spread over 33 and 34 days. The foreign cultivars 'Ohadi', 'Kerman' and 'Red Aleppo' had almost the same median bloom dates (18th–19th April), with the flowering period extending over 26 and 27 days.

Bloom date responses to daily chill and heat accumulation rates were illustrated by combining the two main outputs of the PLS analysis − Variable Importance in the Projection (VIP) and model coefficients − in one plot, for each cultivar (Fig. 1). For daily chill accumulation rates, the PLS model for the 'El-Guetar' cultivar showed mostly important VIP scores (VIP > 0.8; indicated by red or green bars in Fig. 1) coupled with predominantly negative model coefficients between 12th December and 14th March (Fig. 1). During this phase, high daily chill accumulation rates were generally correlated with early bloom dates. We thus interpreted the period from 1st December to 14th March as the chilling period of 'El-Guetar', despite a short period of low VIP scores at the beginning. A similar phase was found for heat accumulation, with high VIP scores and negative model coefficients between 14th March and 14th April (Fig. 1) indicating a correlation between high daily heat accumulation and early bloom dates. This period was interpreted as the forcing period for 'El-Guetar'. Similar reasoning was used to determine the chilling and forcing phases for all other cultivars (Fig. 1 and Table 1).

PLS analysis indicated similar chilling periods for the local cultivars 'Mateur' and 'Nouri', and for the foreign cultivars 'Ohadi', 'Kerman' and 'Red Aleppo'. 'Kerman' and 'Red Aleppo' appeared to have the same forcing phase. Short periods of positive model coefficients and high VIP values (phases shown in green in Fig. 1) were observed during the chilling phases for all pistachio cultivars expect 'Meknassy'. These periods occurred in January and February for 'Mateur', 'Ohadi', 'Kerman' and 'Red Aleppo', in December, January and February for 'El-Guetar' and only in January for 'Nouri'. 'Meknassy' had no periods with positive model coefficients during chill accumulation, but also showed phases of reduced chill efficacy during this period, indicated by temporarily less negative model coefficients. These observations imply that trees vary in their chill responsiveness during the chilling phase, which may even be interrupted by periods when trees are entirely unresponsive to chill.

For 'El-Guetar', during the last 20 days of November, a short period of positive model coefficients and high VIP values was observed, which indicated a later start of the chilling period compared to the other cultivars. We also detected important effects of heat accumulation in September and October, before the start of the chilling period (shown in green in the right panel of Fig. 1). For all cultivars, this phase consisted of a period of approximately two weeks, when high heat accumulation was correlated with late bloom. This phase was followed by a longer period, lasting up to five weeks (though not all daily accumulation values were identified as important), when high heat accumulation was associated with early bloom.

3.2. Chilling and heat requirements

Based on the PLS regression outputs, chilling and heat requirements were estimated by calculating mean chill (using the Dynamic Model) and heat accumulation (with the Growing Degree Hours Model) during the respective periods between 1997 and 2016. Chilling requirements (with ± indicating standard errors of the calculated mean) were between 32.1 ± 2.3 and 33.3 ± 2.2 CP and heat requirements between 9974 ± 198 and 12,738 ± 235 GDH for the various cultivars (Table 1). The local cultivars 'Mateur' and 'Nouri' had the lowest and 'El-Guetar' the highest chilling requirements. All foreign cultivars had the same chilling requirements (32.4 ± 2.3), similar to the local cultivar 'Meknassy'. The highest heat requirement was identified for the foreign cultivar 'Ohadi' (12,738 ± 235) and the lowest for the local cultivar 'El-Guetar' (9974 ± 198). 'Kerman' and 'Red Aleppo' had the same heat requirements (12,374 ± 232).

Table 1
Bloom dates, chilling and forcing periods and chilling and heat requirements (including the standard error of the mean) for local and foreign pistachio cultivars from 1997 to 2016.

Cultivars	First bloom Dates				Chilling period		Forcing period		Chilling requirements	Heat requirements
	First	Last	Range (Days)	Median	Start	End	Start	End	Chill Portions	Growing Degree Hours
El-Guetar	26 Mar	28Apr	33	14 Apr	1 Dec	14 Mar	14 Mar	14 Apr	33.3 ± 2.2	9974 ± 198
Mateur	25 Mar	28Apr	34	10 Apr	17 Nov	4 Mar	7 Mar	10 Apr	32.1 ± 2.3	10,258 ± 223
Meknassy	26 Mar	28Apr	33	13 Apr	17 Nov	5 Mar	8 Mar	13 Apr	32.4 ± 2.3	11,053 ± 229
Nouri	24 Mar	26 Apr	33	10 Apr	17 Nov	4 Mar	6 Mar	10 Apr	32.1 ± 2.3	10,538 ± 229
Ohadi	5 Apr	2 May	27	19 Apr	17 Nov	6 Mar	10 Mar	19 Apr	32.4 ± 2.3	12,738 ± 235
Kerman	5 Apr	2 May	27	18 Apr	17 Nov	6 Mar	10 Mar	18 Apr	32.4 ± 2.3	12,374 ± 232
Red Aleppo	4 Apr	30 Apr	26	18 Apr	17 Nov	6 Mar	10 Mar	18 Apr	32.4 ± 2.3	12,374 ± 232

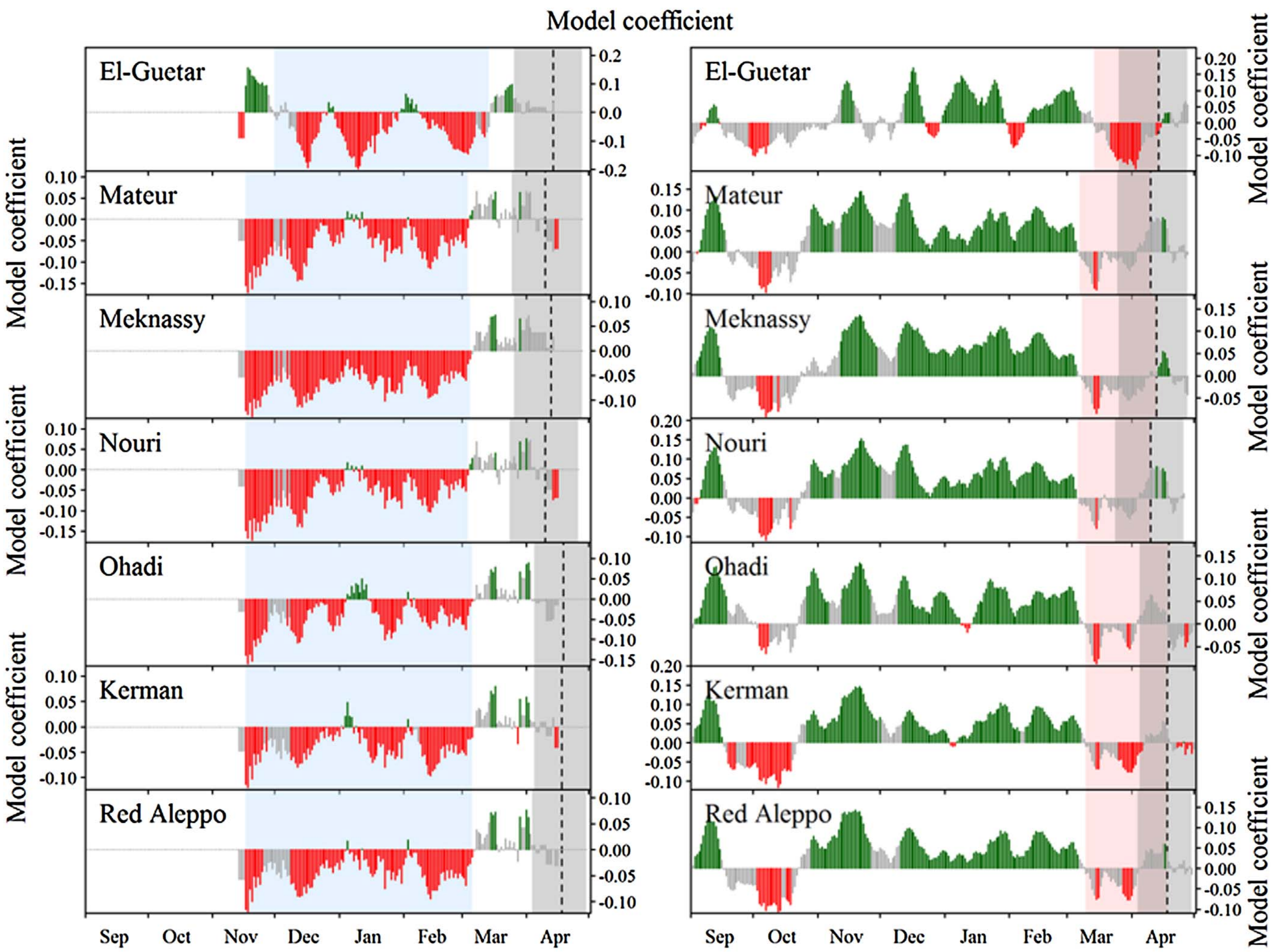

Fig. 1. Model coefficients of Partial Least Squares regression between accumulation rates of agroclimatic metrics (Chill Portions and Growing Degree Hours) and bloom dates of pistachio cultivars. Panels on the left show chill accumulation and panels on the right show heat accumulation. Negative and important model coefficients are marked by red bars and positive and important model coefficients by green bars. The chilling phases are shown by blue shading, and red shading shows the forcing phases. The range of bloom dates is indicated by grey shading, with the dashed lines indicating median bloom dates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

3.3. Bloom date response to temperature during chilling and forcing phases

For all pistachio cultivars, first bloom dates were plotted as a function of mean temperatures during the chilling and forcing phases (Fig. 2). For all cultivars, the near-vertical contour lines in the resulting surface plots implied a strong bloom-delaying effect of high temperature during the chilling phase, compared to a relatively weak bloom-advancing effect of warm conditions during the forcing period.

3.4. Bloom date response to chill accumulation

Bloom dates between 1997 and 2016 showed a clear and highly significant ($p < 0.01$) correlation with chill accumulation (Fig. 3A). Low chill accumulation (between 10 and 23 CP in 2007, 2010 and 2016) had a delaying-effect on bloom dates of 'Mateur' pistachios, leading to bloom dates between 13th and 28th April, as opposed to 24th March to 9th April in years with greater chill accumulation. The latest bloom dates in 2007, 2010 and 2016 followed the three winters with the lowest chill accumulation rates on record (Fig. 4). In 2001, when chill accumulation was 16.3 CP, 'Mateur' trees did not bloom (dashed

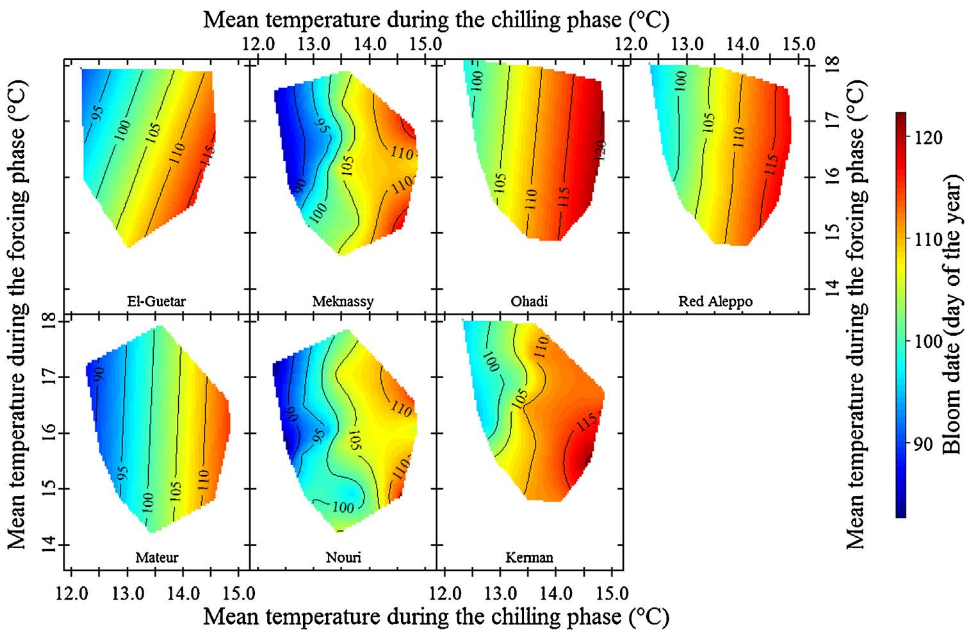

Fig. 2. Response of first bloom dates of pistachio cultivars to mean temperatures during the chilling and forcing phases. Contour lines and the color scheme illustrate bloom dates.

line Fig. 3A). These findings indicate a strong impact of chill accumulation on bloom dates and imply that bloom is delayed or fails altogether, if winter chill falls below a certain threshold.

3.5. Yield response to chill accumulation

Between 1983 and 2015, most cases of low chill accumulation were accompanied by very low yields (< 2 kg/tree) or no production at all for the 'Mateur' cultivar (Fig. 3B). Overall, there was a significant ($p = 0.039$) positive correlation between chill accumulation and yield. All years that were marked by zero production (1995, 2001 and 2007) had chill accumulation between 14 and 26.5 CP, which is below our estimate of the cultivar's chilling requirement (32.1 ± 2.3 CP). Low yields were also observed for several years with higher chill accumulation (around 40 or 50 CP), but these may have been caused by other factors, such as alternate bearing ('off' years), which strongly affects pistachios.

3.6. Effect of chill accumulation on bloom dates for pistachio cultivars

Chill accumulation showed a strong correlation with bloom dates of all tested pistachio cultivars ($p < 0.01$ for all cultivars; Fig. 5). For all cultivars, low chill accumulation (< 30 CP) had a delaying effect on bloom dates. All cultivars failed to bloom in 2001 (Fig. 5), when only 16.3 CP accumulated during the winter. The 'Kerman' cultivar also failed to bloom in 2016, when chill accumulation was 10 CP (Fig. 5). During these years, chill accumulation fell short of meeting chilling requirements, which according to our estimates were between 32.1 ± 2.3 and 33.3 ± 2.2 CP across all cultivars. While yields were only measured for 'Mateur', these findings imply that all cultivars are likely to respond to low chill accumulation with weak or zero production. For 'Mateur', low yields were only loosely (and not statistically significantly at $p = 0.136$) correlated with late bloom dates (Fig. 3C). A clearer correlation was possibly obscured by the influence of other factors, such as alternate bearing (Rosenstock et al., 2010) or rainfall (Elloumi et al., 2013), which are known to cause high variability in pistachio yields.

3.7. Observed symptoms of lack of chill

Field observations in 2015 (Table 2) – after a winter with moderate chill accumulation (36 CP) − showed low bud fall percentages between 2.8 ('Meknassy') and 10% ('Kerman'). All buds of all cultivars broke their dormancy, except for 'Ohadi', for which 5.5% of buds remained dormant. For all foreign cultivars, some buds only reached the budbreak stage, without progressing further towards flowering. This phenomenon was most pronounced for 'Kerman' (43.3% of buds). The highest flowering percentage was reached for 'Meknassy' at 97.2% of all buds, and the lowest for 'Kerman' (31.6%).

In 2016, after a low-chill winter (10 CP, the lowest on record), bud fall percentage was higher than in the previous year, reaching 40.6% for 'Mateur'. Across all cultivars, between 40 and 66% of buds remained dormant. For 'Mateur', 'Ohadi', 'Kerman' and 'Red Aleppo', the development of many buds that had broken dormancy stopped before reaching the flowering stage. The flowering percentage of all cultivars was weak at less than 30%. No buds of 'Ohadi' and 'Kerman' reached the flowering stage, and 'Kerman' buds stopped at the budbreak stage. All 'Kerman' trees in the orchard failed to bloom, while 'Ohadi' trees achieved partial bloom. Bloom periods were longer in 2016 than in 2015 for all cultivars, with inhomogeneous bloom and often several phenological stages coinciding on the same branch due to late and incomplete rest-breaking. Buds that did not reach the flowering stage dried up and fell off. The coincidence of these physiological effects with low chill in 2016 implies that they are symptoms of insufficient chill accumulation.

4. Discussion

Throughout the long-term record of pistachio phenology analysed in this study, local cultivars always bloomed before foreign ones. Bloom timing is determined by cultivar-specific chilling and heat requirements (Luedeling et al., 2009c), with heat accumulation commonly assumed to begin once chilling requirements have been fulfilled (Lang et al., 1987), and bloom occurring when heat needs have also been met (Gao et al., 2012; Richardson et al., 1974). However, there is still uncertainty about when exactly trees start being receptive to heat accumulation (Jones et al., 2015) and about potential interactions between chilling and heat requirements (Gao et al., 2012; Harrington et al., 2010). For precise quantification of climatic needs for dormancy release, however, knowledge on when both chill and heat are effective is essential (Guo et al., 2015b).

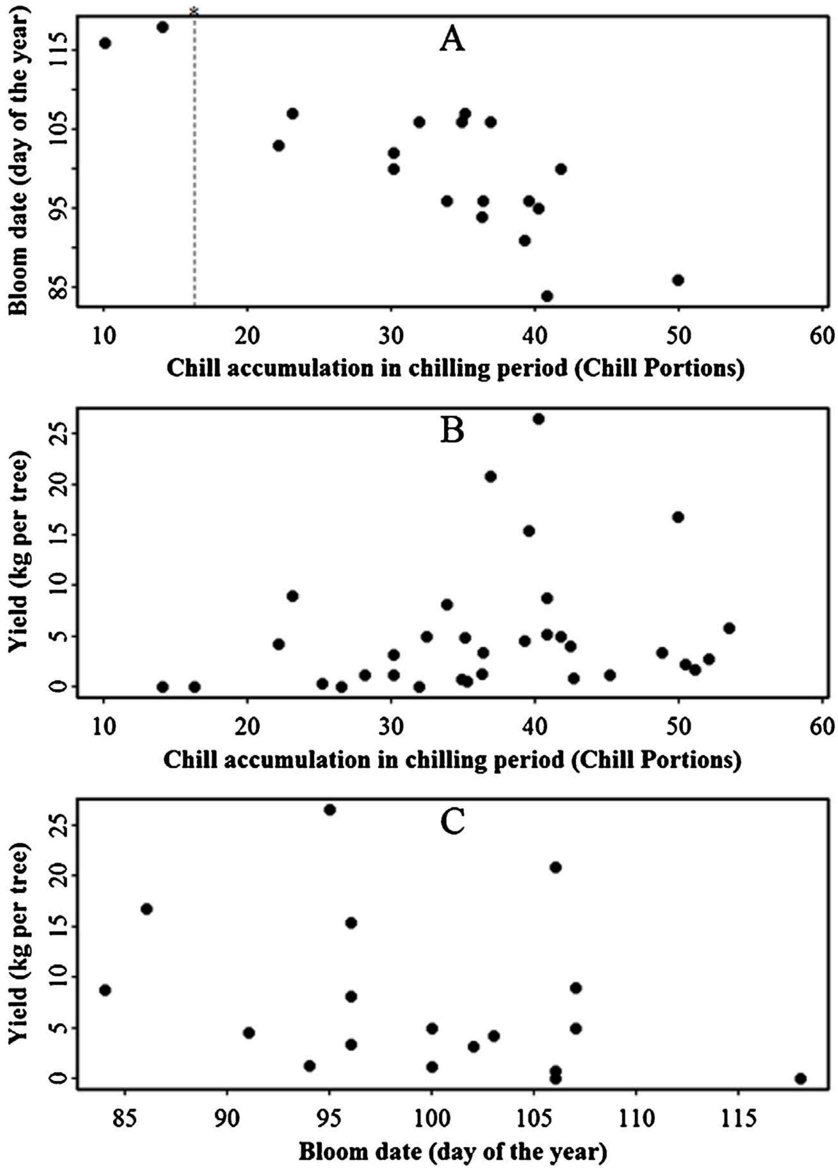

Fig. 3. Relationship of chill accumulation during the chilling phase with bloom dates (A) and yield (B), and of bloom dates with yield (C) for 'Mateur' pistachios in Sfax, Tunisia. The dashed line and * symbol in (A) illustrate chill accumulation in 2001, when trees failed to bloom.

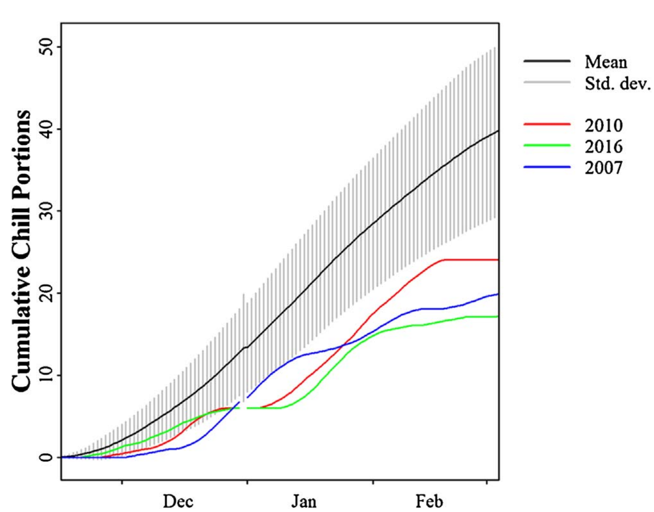

Fig. 4. Historic distribution of cumulative chill accumulation (in Chill Portions) during the chilling phase of the 'Mateur' cultivar, with the three latest-bloom (and lowest-chill) years highlighted. The black line indicates the mean accumulation, while the gray shaded area shows the standard deviation.

4.1. Delineation of temperature response phases

Outputs of the PLS regression suggested that the chilling period of most local and foreign cultivars started on the same day, with only the central Tunisian cultivar 'El-Guetar' lagging two weeks behind. The beginning of the chilling period thus roughly coincided with leaf-fall, which typically occurs in November, and is often considered an indicator of dormancy onset (Kaufmann and Blanke, 2016). Slightly greater variation was found for the beginning of the forcing period and for bloom dates. Interestingly, even though 'El-Guetar' appeared to have a later chilling phase and later start of the forcing period, its bloom date constituted the median of the distribution among all seven cultivars.

PLS outputs for all cultivars showed strong variation in model coefficients during the chilling phase, which in some cases was interrupted by short spells of low VIP scores or even by positive model coefficients for chill accumulation. This suggests that the chilling period may consist of several phases, with differences in trees' temperature responsiveness, as has been suggested for almonds in Sfax (Benmoussa et al., 2017) and for apricots in Beijing (Guo et al., 2015b).

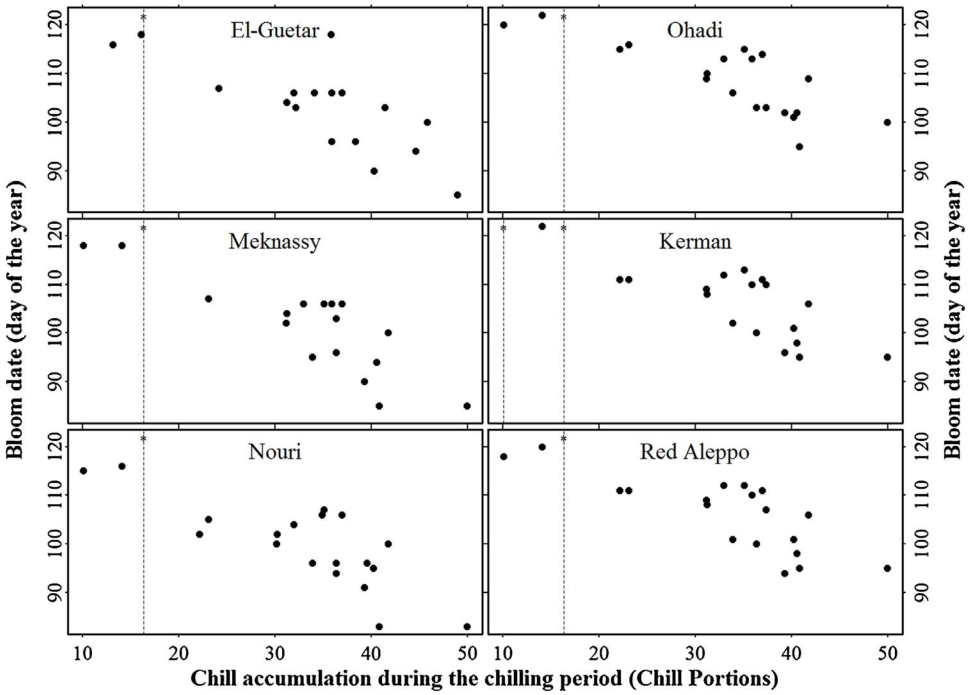

Fig. 5. Response of bloom dates to chill accumulation during the chilling phase of pistachio cultivars. Dashed lines and * symbols indicate years when no bloom occurred (for all cultivars in 2001, and for 'Kerman' also in 2016). A similar plot for 'Mateur' is shown in Fig. 3A.

4.2. Temperature responses during dormancy induction

September and October, the months leading up to dormancy, are warm in Sfax, with around 350 GDH accumulating per day. Yet, consistently across all cultivars, PLS regression indicated a bloom-delaying effect of high heat accumulation in September and a bloom-advancing effect of warm October days (Fig. 1). These results suggest that temperature during dormancy induction affects the dormancy process sufficiently to be reflected in a noticeable effect on bloom dates. This finding implies that short photoperiod is not the only factor that induces dormancy and growth cessation in woody plants, supporting recent work that proposed an important effect of temperature during dormancy induction (Tanino et al., 2010). Effects of temperature on growth cessation and dormancy induction have already been documented for hybrid poplar (Kalcsits et al., 2009) and (in combination with photoperiod) for *Prunus* species (Heide, 2008).

Whether cool temperature or short days are most influential in inducing dormancy appears to vary among species and occasionally even among varieties of the same species (Chao et al., 2007), and it may be related to geographic origin (Tanino et al., 2010). For hybrid aspen, effects of both factors have been demonstrated (Welling et al., 2002), while for apple, pear and *Sorbus* species, only a low-temperature effect has been found (Heide, 2011; Heide and Prestrud, 2005). Tanino et al. (2010) reported that dormancy may be induced by two distinct mechanisms, involving both high and low temperature, and that these mechanisms may co-occur in late summer or autumn. They hypothesize that, even though cool conditions are normally responsible for inducing dormancy, shortening days may also make trees fall dormant in years when temperatures fall later than usual. The combination of these two mechanisms may ensure that trees avoid frost damage in years with gradual onset of cool conditions, as well as in years with warm autumns followed by sudden drops in temperature.

4.3. Chilling and heat requirements of pistachio cultivars in Tunisia

Chilling requirements were between 32.1 ± 2.3 and 33.3 ± 2.2 CP and heat requirements between 9974 ± 198 and 12,738 ± 235 GDH for the various cultivars. The literature does not offer many values for direct comparison, except for an estimate of 36 CP for 'Mateur' at Sfax reported by Elloumi et al. (2013), which was comparable to the 32.1 ± 2.3 CP we determined. For pistachios grown in other locations, chilling requirement estimates with the Dynamic Model are rare, but the numbers that have been published are poorly aligned with our results. Working on 'Kerman' in California, Pope et al. (2014) found a requirement of 69 CP (converted from Ferguson et al. (2008)), and Zhang and Taylor (2011) reported that 'Sirora' pistachios require 58–62 CP in Australia. None of these requirements would be fulfilled in Sfax, even though 'Kerman', which we found to require 32.4 ± 2.3 CP, is successfully produced.

Several estimates are available from different regions and

Table 2
Percentages of buds in the dormant and budbreak stages, percentage of bud fall and percentage of flowering for six pistachio cultivars near Sfax, Tunisia in 2015 and 2016.

Cultivars	2015				2016			
	Bud fall (%)	Bud in dormant stage (%)	Bud in budbreak stage (%)	Flowering (%)	Bud fall (%)	Bud in dormant stage (%)	Bud in budbreak stage (%)	Flowering (%)
Mateur	3.9	0.0	0.0	94.1	40.6	42.2	1.5	15.6
Meknassy	2.8	0.0	0.0	97.2	37.7	39.6	0.0	22.6
Nouri	9.4	0.0	0.0	88.8	15.6	65.6	0.0	18.7
Ohadi	5.5	5.5	1.8	83.3	18.7	64.0	15.6	0.0
Kerman	10	0.0	43.3	31.6	0.0	42.8	57.1	0.0
Red Aleppo	6.7	0.0	33.3	45.0	8.9	43.3	23.9	22.4

determined with models that have proven unreliable when used across locations (Luedeling and Brown, 2011). 'Ohadi' has been reported to require 1050 Chilling Hours and 900 Chill Units in Turkey (Küden et al., 1995) and 1000–1250 Chilling Hours in Iran (Rahemi and Pakkish, 2009). For several cultivars, chilling requirements of 1000–1400 Chilling Hours have been reported for Iran (Afshari et al., 2009; Esmaeilizadeh et al., 2006). These requirements are clearly not valid in Tunisia, where this many Chilling Hours or Chill Units are never experienced, but many of the studied cultivars are grown. It is also noteworthy that in the Sfax region, the 'Ohadi' cultivar was more productive than 'Mateur' over a fifteen year period (Ghrab et al., 2005), even though according to estimates from Turkey and Iran, which used the Chilling Hours model, its chilling requirement is much greater.

All studied cultivars had roughly the same chilling requirements. This implies that options exist for increasing the diversity of Tunisian pistachio orchards, which are currently dominated by 'Mateur'. This may also make it relatively easy to use other cultivars in breeding programs aiming at quality characteristics or resistance/tolerance to abiotic and biotic stresses. Variation among heat requirements (9974 ± 198 and 12,738 ± 235 GDH) induced differences in bloom dates. While we only investigated female cultivars, considering synchrony between male and female cultivars is required for orchard managers, because of the importance of cross-fertilization. Differences in chilling and heat requirements between male and female cultivars and temperature changes may cause asynchronous flowering in currently productive cultivar pairs, which could lead to poor pollination. For example, the male cultivars 'A25' and 'A40' are often selected as pollinators for 'Mateur' in northern Tunisia, but they are unable to fulfil this function in central Tunisia (Ghrab et al., 2002), because of flowering asynchrony between the cultivars.

Compared to chilling requirements, our estimates of heat requirements were relatively well aligned with numbers reported from elsewhere. For 'Mateur', the heat requirement we determined (10,258 ± 223 GDH) was less than 20% below the 12,000 GDH reported by Salhi et al. (2014). For 'Ohadi' we estimated 12,738 ± 235 GDH, while Küden et al. (1995) found 11,500 GDH and Rahemi and Pakkish (2009) between 12,871 and 13,320 GDH. While these results are relatively similar, the remaining differences could be explained by the use of cuttings in laboratory settings (Rahemi and Pakkish, 2009; Salhi et al., 2014), as opposed to our field observations, and slight differences in the GDH model (the three mentioned studies used the GDH-Utah model (Richardson et al., 1975), while we used the GDH model proposed by Anderson et al. (1986)).

4.4. Warm chilling periods delay bloom

Temperature response analyses for all cultivars showed a strong bloom-delaying effect of high temperatures during the chilling phase, while warm conditions during the forcing period caused only small advances in bloom dates. This finding is similar to earlier results for almond and pistachio in Sfax (Benmoussa et al., 2017; Elloumi et al., 2013), for peach in Mornag in northern Tunisia (Ghrab et al., 2014a,b) and for apple in France (Legave et al., 2013). Most authors, however, expect the dominant temperature response to be an advance in bloom dates caused by warm forcing conditions. For instance, Guédon and Legave (2008) estimated that global warming could cause bloom advances of 7–8 days for apple and 10–11 days for pear.

It has been noted that bloom date responses to temperature may vary considerably across different regions (Menzel and Sparks, 2006). Working on apricots in China along a climate gradient involving five locations, Guo et al. (2015a) detected a strengthening of the bloom-delaying effect of warm chilling periods with increasing temperatures during this part of the year. While at the coldest location in their study, chilling phase temperatures barely had any effect on bloom timing, bloom dates at the warmest site appeared to be driven by chilling and forcing temperatures in approximately equal measure. Our results on pistachio in Sfax imply that this trend continues as temperatures increase past the range covered by Guo et al. (2015a), towards a near-exclusive effect of chilling phase temperature in determining bloom dates. The Sfax region is currently considered a marginal pistachio growing region, in terms of the trees' ability to fulfill their chilling requirements. Increasing temperatures during the winter may exacerbate this risk, causing delays in the breaking of bud dormancy, which in turn may lead to late flowering and ultimately low yields.

Pistachio bloom dates were strongly correlated with chill accumulation, with low chill (between 10 and 23 CP in 2007, 2010 and 2016) delaying bloom dates of all cultivars. This phenomenon was also observed by Elloumi et al. (2013) for pistachio and by Ghrab et al. (2014a,b) for peach in 2007 and 2010, and it was almost certainly caused by lack of chill (Atkinson et al., 2013). In 2001, when chill accumulation was 16.3 CP, none of the cultivars received sufficient chill to flower, and 'Kerman' showed no bloom also in 2016 (after receiving 10.0 CP). These findings indicate that pistachio cultivars are unable to bloom, when annual chill falls below a certain threshold.

4.5. Yield response to lack of chill

Between 1983 and 2015, low chill accumulation was correlated with very low yields (< 2 kg/tree) or no production at all for the 'Mateur' cultivar. While Pope et al. (2014) showed that bud-based requirements did not affect the nut yield of 'Kerman' in California, we detected a strong effect for Tunisia, with zero production in 1995, 2001 and 2007 after winters that failed to meet the cultivar's chilling requirement. The main mechanism involved was a delay in bloom dates, which affected pollination, pollen quality and viability, and subsequent fruit set. In 1997, 2001, 2007, 2010 and 2013, Ghrab et al. (2016) observed that pistachio and almond in the Sfax region were increasingly exposed to lack of chill. In addition, during 2007 and 2010, for peach in Mornag (northern Tunisia), Ghrab et al. (2014a,b) observed unusually long flowering periods with very low yields. Several reported symptoms of insufficient chill, including irregular or delayed budbreak, abscission of floral buds, reduced flower quality and poor fruit set (Atkinson et al., 2013), may be responsible for crop failures, which were observed in 'Mateur' and likely also occurred in other pistachio cultivars. During field observations in 2016, after a warm winter with a record-low 10 CP, trees showed many signs of insufficient chill, including low flowering percentage, high percentage of bud fall, long and inhomogeneous bloom, and co-occurrence of several phenological stages on the same branch. The same phenomena were detected for almond (Ghrab et al., 2016) and peach (Ghrab et al., 2014b) in 2007 and 2010. For almond, many flower buds failed and physiological disorders were observed (double and triple fruits for the 'Tuono' cultivar), while for peach, phenological stages were heterogeneous. Fruits remained small, leaves were scattered, extra fruit growth occurred, some fruits grew in pairs, fruit size was unusually variable, and some fruits were triangular and without stone.

4.6. Limitations of the dynamic model

While the Dynamic Model has often been found to be the most suitable among common chill models, especially in warm regions, it does not seem well suited for the warm climate of Sfax. Regarding dormancy induction, it is noteworthy that for all cultivars except 'El-Guetar', the beginning of the chilling period coincided almost exactly with the day the first Chill Portion accumulated, and that PLS regression indicated a strong effect for these earliest Chill Portions. The absence of an initial period when Chill Portions accrued but were not yet effective may indicate that the Dynamic Model did not capture chill accumulation accurately enough to detect all effective chill under the warm conditions of Tunisia. Similarly, Benmoussa et al. (2017) found that with the Dynamic Model it was difficult to determine the beginning of the chilling period for many almond cultivars in Sfax. Moreover, our observation that most pistachio cultivars bloomed after receiving 10 CP but not after 16.3 CP raises some questions about the accuracy of the Dynamic Model in capturing the biological processes. The stark differences between chilling requirements determined for ‘Kerman’ in California (69 CP) and the ones identified in the present study (32.4 ± 2.3 CP) also hint at limited applicability of the Dynamic Model across different climates.

4.7. Cross-regional implications

Our observations are compelling evidence that yield losses and deterioration of fruit and nut quality can result from lack of chill. These findings may have implications for production sites that currently have cooler winters, but where global warming is expected to strongly impact production conditions in the future. Such regions include California, Iran and Turkey, which currently generate the bulk of the global pistachio production. If climate change reduces winter chill to levels currently observed in Tunisia, production in many growing regions may be at risk. Our work draws attention to the inadequacy of prevailing chill models, and to the need to close knowledge gaps related to the timing of chilling and forcing phases. It also emphasizes the importance of developing new models for quantifying chill that perform well in warm (and warming) growing regions. For Sfax, our work highlights the chill-related risks that growers using the current array of cultivars face at present and in the future. New strategies to keep the pistachio industry viable are urgently needed.

5. Conclusions

Under the warm conditions in Sfax, pistachios are barely able to satisfy their chilling requirements, causing production problems in years following warm winters. This finding underscores the impression that Sfax is a marginal area for pistachio. Unlike what has been reported for many locations around the world, bloom dates were mainly driven by temperature conditions during the chilling period, with warm winters leading to late bloom. In addition to these effects, we detected a possible influence of temperature conditions during dormancy induction on bloom dates. This effect requires further scientific attention, including physiological studies to elucidate how summer and early autumn conditions affect dormancy progression. Our findings confirm that orchards in warm Mediterranean climates that feature temperate-zone species are under threat from climate change. They also indicate that no common chill models − not even the Dynamic Model, which has performed well in other warm growing regions − are well suited for the warm Tunisian climate. This implies an urgent need to develop suitable chill and phenology models for warm conditions and to develop adaptation strategies to ensure orchard viability in a warming future.

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